

**CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH**

**SUMMARY OF TOXICOLOGY DATA
CYAZOFAMID**

(4-chloro-2-cyano-N,N-dimethyl-5-(4-methylphenyl)-1H-imidazole-1-sulfonamide)

Chemical Code # 5930, Document Processing Number (DPN) # 52995

SB 950 # New A.I.

Original: 7/27/06

Revised: 2/5/07

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effect indicated
Chronic toxicity, dog:	No data gap, no adverse effect indicated
Oncogenicity, rat:	No data gap, no adverse effect indicated
Oncogenicity, mouse:	No data gap, no adverse effect indicated
Reproduction, rat:	No data gap, no adverse effect indicated
Teratology, rat:	No data gap, no adverse effect indicated
Teratology, rabbit:	No data gap, no adverse effect indicated
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Study not required at this time

Toxicology one-liners are attached.

All record numbers for the above study types through 228631 (Document No. 52995-0133) were examined.

In the 1-liners below:

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

indicates a study on file but not yet reviewed.

File name: T060727A.wpd

Revised by T. Moore, 7/27/06; P. Leung, 2/5/07

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may identify additional effects.

COMBINED, RAT

** 0030, 220221; "IKF-916 24-Month Oral Chronic Toxicity Study and Oncogenicity Study in Rats" (Nakashima, N., The Institute of Environmental Study, Tokyo, Japan, Report No. IET 95-0079, 06/07/99). 835. Technical grade IKF-916 (Lot no. 9506, purity = 95.5%) was admixed to the basal diet and fed to 85 Fischer (F344/DuCrj) rats per sex per dose at dose levels of 0, 10 (males only), 50, 500, 5000, or 20000 (females only) ppm (0, 0.336, 1.681, 17.07, and 171.1 mg/kg/day, respectively, for males and 0, 2.010, 20.24, 207.8, and 856 mg/kg/day, respectively, for females) for 104 weeks. 50 animals per sex per dose were used in the main group and 35 animals per sex per dose were used in the satellite group. In the satellite group, 10 animals from each sex were sacrificed at weeks 26, 52, and 78. At least 70% of the main group animals at all dose levels (both sexes) were living at week 104. Mortalities were independent of dose. A treatment-related decrease in body weight was observed in females at 5000 and 20000 ppm during treatment. Hematology investigations revealed no toxicologically significant effects. Serum chemistry investigations revealed a treatment-related decrease in mean triglyceride level in males (week 26, at 5000 ppm) and females (week 52, at 5000 and 20000 ppm). Urinalysis revealed a treatment-related increase in mean urine volume in males (week 77, at 5000 ppm) and females (weeks 51 and 77, at 20000 ppm). A statistically significant increase in mean relative kidney weight in males (week 26, at 5000 ppm) and in females (weeks 26 and 78, at 5000 and 20000 ppm) and a statistically significant increase in mean relative liver weight in males (week 26, at 5000 ppm) and in females (week 26, at 20000 ppm) were observed. Necropsy findings are considered incidental. Microscopic examination revealed no treatment-related neoplasms and no treatment-related non-neoplastic lesions. **No adverse effects.** NOEL (M) = 17.07 mg/kg/day (500 ppm) based on increased mean relative liver and kidney weights and (F) = 20.24 mg/kg/day (500 ppm) based on decreased body weight and increased mean relative liver and kidney weights. **Acceptable.** (Corlett and Leung, 05/01/06)

CHRONIC TOXICITY, RAT

See Combined, Rat above.

CHRONIC TOXICITY, DOG

** 0025, 220215; "A 52-Week Oral Toxicity Study in Dogs with Technical IKF-916 (Includes Report Amendment No. 1)" (Savides, M.C. and M. Watson, Department of Toxicology, Ricerca, Inc., Painesville, OH, Document Numbers 7055-96-0273-TX-002 and 7055-96-0273-TX-002-001, 03/21/01). 831. Technical grade IKF-916 (Lot no. 9506, purity = 96.4%) was administered orally by capsule(s) daily to 6 beagle dogs per sex per dose at dose levels of 0 (empty capsules), 40, 200, or 1000 mg/kg/day for 52 consecutive weeks. No mortalities occurred. No effects on body weight were observed. Hematology and clinical chemistry investigations revealed no toxicologically significant effects. Urinalysis revealed no treatment-related effects. Organ weight data revealed no biologically significant effects. Necropsy revealed no treatment-related abnormalities. Microscopic examination revealed no treatment-related histopathological findings. **No adverse effects.** NOEL (M/F) = 1000 mg/kg/day based on no effects at the highest dose tested. **Acceptable.** (Corlett and Leung, 04/04/06)

ONCOGENICITY, RAT

See Combined, Rat above.

ONCOGENICITY, MOUSE

** 0026, 220217; "An Oncogenicity Study in Mice with Technical IKF-916" (O'Meara, H. and Watson, M., Toxicology and Metabolism, Ricerca, Inc., Painesville, OH, Report No. 6785-96-

0071-TX-003, 05/07/99). 832. Technical IKF-916 (Lot no. 9506, purity = 95.5%) was admixed to the basal diet and fed to 60 CrI:CD-1® (ICR) BR VAF/Plus® mice per sex per dose at dose levels of 0, 0, 70, 700, or 7000 ppm (0, 0, 9.5, 94.8, and 984.9 mg/kg/day, respectively, for males and 0, 0, 12.2, 124.3, and 1203.4 mg/kg/day, respectively, for females) for 18 consecutive months. Percentage of animals surviving to the termination of the study at each dose level was as follows- males: 82%, 85%, 78%, 87%, and 78%, respectively; females: 83%, 77%, 78%, 77%, and 77%, respectively. No toxicologically significant clinical signs were observed. No treatment-related effects on body weight were observed. Mean differential leukocyte investigations revealed no treatment-related effects. Hematology and serum chemistry investigations revealed no toxicologically significant effects. Organ weight data revealed no toxicologically significant effects. Necropsy revealed no treatment-related effects. Microscopic examination revealed no treatment-related neoplasms and no treatment-related non-neoplastic lesions. **No adverse effects.** NOEL (M) = 984.9 mg/kg/day (7000 ppm) and (F) = 1203.4 mg/kg/day (7000 ppm) based on no effect at the highest dose tested. **Acceptable.** (Corlett and Leung, 05/16/06)

REPRODUCTION, RAT

**52995-029, 0131, 0132, 0133, 220220, 228629, 228630, and 228631; "A Two-Generation Reproduction Study in Rats with Technical IKF-916" (Helen M. O'Meara and Michael Watson, Ricerca, Inc., Toxicology and Metabolism, Painesville, OH., Report # 6755-96-0042-TX-003, 24 November 1998). Thirty pairs of CrI:CD® BR VAF/Plus® rats per generation were dosed via the diet with Cyazofamid (IKF-916 Technical), Batch 9506, purity 95.5% at 0, 200, 2000, or 20000 ppm. Treatment was continuous for 2 generations, with 2 littering periods per generation. Mean compound intake by F0 and F1 rats at pre-mating period week 10 averaged 11.2, 112, and 1163 mg/kg/day for males, and 14.5, 142, and 1488 mg/kg/day for females. Parental NOEL = 2000 ppm, based on slightly reduced bodyweights in F1 20000 ppm parents. Also, bodyweights of F0 and F1 dams during gestation and lactation were reduced (often significantly) at 20000 ppm. Food intake was not affected by treatment. Offspring NOEL = 2000 ppm [significantly reduced pup weights (averaging 4 g) in all littering periods at 20000 ppm by PND 21]. Original DPR review (Aldous, 6/26/06) found the study not acceptable but upgradeable (estrous staging information was requested, along with recent (within 2 years of current study) historical control data from contemporary reproduction studies including numbers of stillborn pups/litter and percentages of litters with stillborn pups). No adverse effect was indicated. Estrus staging information and a preliminary one-generation reproduction study (used for dose selection) were submitted. Treatment-related effects on stillborn parameters are not indicated. The study is upgraded to acceptable. (Green and Leung, 2/1//07).

52995-0132 228630, "A One-Generation Reproduction Study in Rats with IKF-916", (Helen M. O'Meara, *et al.*, Ricerca, Inc., Toxicology and Animal Metabolism, Painesville, OH., Report # 6754-96-0041-TX-001, 30 December 1996). 10 Sprague-Dawley CrI:CD® BR VAF/Plus® rats per sex per group received IKF-916 Technical in the diet at 0 (untreated diet), 1000, 3000, 7000, and 20000 ppm through production of 1 litter. Treatment began 8 weeks prior to mating. Average IKF-916 Technical intake for weeks 1 through 8 was 66.5, 200, 450, and 1327 mg/kg/day for males and 77.5, 252, 562, and 1613 mg/kg/day for females at 1000, 3000, 7000, and 20000 ppm respectively. Two high dose females were sacrificed moribund around the time of parturition (both had pups *in utero*). There were no treatment-related effects on bodyweight, bodyweight gain, or food consumption of parental animals. Ten, 10, 9, 8, and 7 live litters resulted at 0, 1000, 3000, 7000, and 20000 ppm respectively. There were no treatment-related effects on pups or litters. Parental and Reproductive NOEL = 20000 ppm. No adverse effects. Necropsies and histopathology were not performed. Supplemental data. (Green, 12/12/06).

TERATOLOGY, RAT

** 0027, 220218; "IKF-916 Technical: A Developmental Toxicity Study in the Rat Via Oral Administration" (Rodwell, D.E., Huntingdon Life Sciences Ltd., East Millstone, NJ, Study No. 98-4149, 06/11/99). 833. IKF-916 Technical (Lot no. 9506, purity = 95.5%) was administered as a single daily dose by gavage to 25 pregnant VAF/Plus® Sprague Dawley - derived (CD®) [Crl: CD® BR] rats per dose at dose levels 0 (0.5% (w/v) aqueous methylcellulose), 30, 100, or 1000 mg/kg/day from gestation day 0 through gestation day 19. No maternal deaths were observed. No treatment-related effects on body weight or food consumption were observed. No treatment-related clinical signs were observed during gestation. Macroscopic examination of the dams revealed no treatment-related abnormalities. Analyses of mean fetal weight, mean number of fetuses per animal, and the mean number of resorptions per animal revealed no treatment-related effects. No treatment-related fetal abnormalities were observed. **No adverse effects. Maternal NOEL and Developmental NOEL = 1000 mg/kg/day** (based on no effects at the highest dose tested). **Acceptable.** (Corlett and Leung, 06/08/06)

TERATOLOGY, RABBIT

** 0028, 220219; "IKF-916 Technical: A Developmental Toxicity Study in the Rabbit Via Oral Administration" (Rodwell, D.E., Huntingdon Life Sciences Ltd., East Millstone, NJ, Study No. 98-4150, 06/11/99). 833. IKF-916 Technical (Lot no. 9506, purity = 95.5%) was administered as a single daily dose by gavage to 24 pregnant New Zealand White rabbits per dose at dose levels 0 (0.5% (w/v) aqueous methylcellulose), 30, 100, or 1000 mg/kg/day from gestation day 4 through gestation day 28. One female at 100 mg/kg/day aborted and was sacrificed on day 26 of gestation; this animal had delivered 1 fetus and 10 fetuses were noted *in utero*. One female at 1000 mg/kg/day aborted and was sacrificed on day 27 of gestation; this animal had delivered 2 fetuses and 7 fetuses were noted *in utero*. No treatment-related effects on body weight or food consumption were observed. No treatment-related clinical signs were observed during gestation. Macroscopic examination of the dams revealed no treatment-related abnormalities. Analyses of mean fetal weight, mean number of fetuses per animal, and the mean number of resorptions per animal revealed no treatment-related effects. No treatment-related fetal abnormalities were observed. **No adverse effects. Maternal NOEL and Developmental NOEL = 1000 mg/kg/day** (based on no effects at the highest dose tested). **Acceptable.** (Corlett and Leung, 06/15/06)

GENE MUTATION

**52995-0031 220222 Kitching, J., "IKF-916 Technical: Bacterial mutation assay," Huntingdon Life Sciences Ltd., 4/30/98. ISK Document # RIA 005/982367. A reverse-mutation study was undertaken using 4 Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, plus Escherichia coli strain CM891 with Cyazofamid (IKF-916 Technical), 95.5% in acetone as test article. There were two independent trials (one by plate incorporation and one by 30 min pre-incubation, each followed by further incubation at 37 °C for 72 hours) with three replicates/dose with and without S9 at dose levels of at least 5000, 1500, 500, 150, and 50 in both trials. There were no increased revertants in either trial due to treatment, with or without activation. Precipitation occurred at 1500 µg/plate and up in the plate incorporation tests, and at 500 µg/plate and up in the pre-incubation tests. Positive controls were functional. Study is acceptable, with no adverse effects. Aldous July 3, 2006.

**52995-0036 220227 Kirkpatrick, D., "IKF-916 Technical: mammalian cell mutation assay," Huntingdon Life Sciences Ltd., 11/16/98. ISK Document # RIA 008/982368. L5178Y mouse lymphoma cells were exposed to 0, 25, 50, 75, and 100 µg/ml Cyazofamid (IKF-916 Technical), Batch 9506, purity 95.5%, in a study to assess forward mutation to thymidine kinase deficient phenotype. Following a 3-hr exposure to test article, cells were washed, re-suspended, and maintained for 2 days in culture tubes for expression. Contents were then adjusted to about 1.6 cells/100 µl aliquot, divided to well plates, and allowed 7 days for expression. Investigators used two cultures per dose level per test, and there were two independent tests with S-9 and

two tests without S-9 at each of the above levels. The highest dose level caused precipitation and a reduction of relative survival to 35-46% without S-9 and to 46 to 48% with S-9 (evidence of toxicity, but valid for interpretation). Results did not meet criteria for a treatment effect. Positive controls were functional. The study is negative for mutagenicity. **Acceptable, with no adverse effects.** Aldous, July 3, 2006.

Supplementary studies not testing the active ingredient:

52995-0032 220223 Matsumoto, K., "CCIM: Reverse mutation test," The Institute of Environmental Toxicology, Tokyo, Japan, 3/17/99. ISK Document #: IET 98-0090. This study evaluated mutagenicity of CCIM (4-chloro-5-p-tolylimidazole-2-carbonitrile), a key metabolite of cyazofamid, purity 99.0%, in a reverse-mutation study using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100, and *Escherichia coli* strain WP2 uvra. There was a single trial, with two replicates per dose level. Investigators used the pre-incubation method (20 min). Exposure levels were in 2-fold steps, with the highest level at 1250 µg/plate. Dose levels above 313 or 625 µg/plate had excessive toxicity, so that only levels up to 156 or 313 µg/plate were usable in the assay. Positive controls were functional. Useful supplementary data. No adverse effects. Aldous, July 3, 2006.

52995-0033 220224 Matsumoto, K., "CTCA: Reverse mutation test," The Institute of Environmental Toxicology, Tokyo, Japan, 4/19/99. ISK Document #: IET 98-0092. This study evaluated mutagenicity of CTCA (4-chloro-5-p-tolylimidazole-2-carboxylic acid), a metabolite of cyazofamid, purity 99.7%, in a reverse-mutation study using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100, and *Escherichia coli* strain WP2 uvra. There was a single trial, with two replicates per dose level. Investigators used the pre-incubation method (20 min). Exposure levels were in 2-fold steps, with the highest tolerated level at 2500 µg/plate in *Salmonella* or 5000 µg/plate in *E. coli*. Useful supplementary data. No increased reversions: no adverse effects. Aldous, July 3, 2006.

52995-0034 220225 Matsumoto, K., "CCIM-AM: Reverse mutation test," The Institute of Environmental Toxicology, Tokyo, Japan, 4/19/99. ISK Document #: IET 98-0091. This study evaluated mutagenicity of CCIM-AM (4-chloro-5-p-tolylimidazole-2-carboxamide), a metabolite of cyazofamid, purity 99.6%, in a reverse-mutation study using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100, and *Escherichia coli* strain WP2 uvra. There was a single trial, with two replicates per dose level. Investigator used the pre-incubation method (20 min). In the range-finding test, exposures up to 5000 µg/plate caused little or no toxicity. Due to precipitation observed at 1250 and 5000 µg/plate, the highest level in the primary test was 1250 µg/plate, with lower levels in 2-fold steps. Precipitation was observed at 625 and 1250 µg/plate without S-9 and at 1250 µg/plate with S-9 in the primary test. Useful supplementary data. No increased reversions: no adverse effects. Positive controls were functional. Aldous, July 3, 2006.

52995-0035 220226 Matsumoto, K., "DMSA: Reverse mutation test," The Institute of Environmental Toxicology, Tokyo, Japan, June 8, 1999. ISK Document #: IET 99-0027. This study evaluated mutagenicity of DMSA (dimethylsulfamic acid), a metabolite of cyazofamid, purity 99.0%, in a reverse-mutation study using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100, and *Escherichia coli* strain WP2 uvra. There was a single trial, with two replicates per dose level. Investigators used the pre-incubation method (20 min). In the range-finding test, exposures up to 5000 µg/plate caused no toxicity nor precipitation. The highest level in the primary test was thus 5000 µg/plate, with lower levels in 2-fold steps. No increased revertant colonies. Positive controls were functional. Useful supplementary data. Aldous, July 3, 2006.

CHROMOSOME EFFECTS

**52995-0037 220228 Akhurst, L. C., "IKF-916 Technical: *in vitro* mammalian chromosome aberration test in human lymphocytes," Huntingdon Life Sciences Ltd., Cambridgeshire, June 11, 1998. ISK Document # RIA 006/982370. Lymphocytes were assessed from whole blood cultures to assess Cyazofamid (IKF-916 Technical), Batch 9506, purity 95.5%. There were two metaphase tests, each with and without S-9 addition. Concentrations of cyazofamid in the first test (with and without S-9) were 0 (acetone vehicle), 50, 100, and 200 µg/ml. The second test with S-9 used the same dose levels. The second test without S-9 used dose levels of 0 (acetone vehicle), 50, 100, and 150 µg/ml. There were duplicate culture tubes for each test/S-9 combination, with 100 metaphase cells evaluated per culture tube. Relative mitotic index estimates for 200 µg/ml groups were less than 50% with and without S-9 in both trials. There were no significant changes at any dose level in aberrant cell counts in either trial, with or without S-9 (pp. 21, 22, 25, and 26). There were fully functional positive controls in all cases. Historical incidence of aberrant cells (excluding gaps) was 0.59% without S-9 and 0.57% with S-9 compared to 0 to 1.5% in treated groups in this study. Positive control incidences in this study corresponded well to historical incidences for the testing lab. Gap incidences were reported, but were of low incidence and without indication of treatment effects. No adverse effects. Acceptable. Aldous, 5/26/06.

52995-0038 220229 Proudlock, R. J. and I. S. Dawe, "IKF-916 Technical: mouse micronucleus test," Huntingdon Life Sciences Ltd., 11/16/98. ISK Document # RIA 007/983715. CD-1 mice, 5/sex/group, were dosed once by gavage with 0, 500, 1000, or 2000 mg/kg cyazofamid, then bone marrow smears were examined for micronucleated immature erythrocytes at 24 hr. Additional control and 2000 mg/kg mice were examined at 48 and 72 hr (5/sex/dose/time). Positive controls were dosed by gavage with 12 mg/kg Mitomycin C, 24 hr before sacrifice. Mice tolerated the treatment with cyazofamid, with no clinical signs or deaths at 2000 mg/kg. Study was negative for micronucleus frequency responses. Positive controls were functional. **Acceptable, with no adverse effects. Aldous, 6/26/06.

DNA DAMAGE

52995-0039 220230 Akanuma, M., "IKF-916 Technical: DNA repair test (rec-assay)," The Institute of Environmental Toxicology, Tokyo, Japan, 8/26/98. ISK Document # IET 98-0053. *Bacillus subtilis* strains H17 [rec+ (wild type)] and M45 [recE⁻ (recombination-deficient)] were used in a standard rec-assay. There were duplicate plates for each dose level with and without S-9 for each strain at treatment levels of 0, 250, 500, 1000, 2000, 4000, and 8000 µg/disk of cyazofamid (IKF-916 Technical), Lot No. 9506, purity 95.5%. Negative control was kanamycin. Positive controls were Mitomycin C (0.01 µg/disk, without S-9) and 3-amino-1,4-dimethyl-5H-pyrido[4,3-b] indole (5 µg/disk, with S-9). **Unacceptable:** there was no analysis of dosing materials, and no alternative evidence (such as precipitation) to demonstrate that maximal practicable dose levels were achieved. There were no inhibitory zones in the two strains at any dose level, with or without S-9. Positive controls were functional. Aldous, 6/26/06.

[NOTE: Mouse micronucleus assay (see Chromosomal aberration section above) has been used to cover DNA Damage data requirements].

NEUROTOXICITY

Acute Neurotoxicity Study

0040, 0041, 51746-0049; 220231, 220232, 221502; "An Acute Neurotoxicity Study in Rats with IKF-916 Technical" (Ridder, W.E. et al., Toxicology & Metabolism, Ricerca, LLC, Painesville, OH, Study Number (referred to as Document Number) 8088-1, 12/21/00). 818. IKF-916 Technical (Lot No. 9506, purity= 95.7%), suspended in 0.5% (w/v) aqueous methylcellulose, was administered in a single dose by gavage to 10 Sprague-Dawley (CrI:CD[®] (SD)IGS BR[®]) rats per sex per dose at dose levels of 0 (vehicle control), 80, 400, or 2000

mg/kg. No mortalities occurred. Cageside observations and weekly physical examinations revealed no treatment-related clinical signs. No biologically-significant effects on body weight or body weight gain were observed. No treatment-related effects on mean grip strength and mean landing foot spread were observed. FOB observations and motor activity (mean distance and mean rest time measured) assessments revealed no treatment-related effects on the day of treatment (approximately 30-60 minutes after treatment), and 7 and 14 days after treatment. Macroscopic and microscopic examinations revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M/F) = 2000 mg/kg (based on no effects at the highest dose tested). **Unacceptable but possibly upgradable** with the submission of more recent motor activity positive control data and with the submission of positive control data supporting the sensitivity of FOB evaluations. (Corlett and Leung, 03/08/06)

METABOLISM

****52995-0045 220236** McFadden, J. J., M. Yoshida, and K. L. Huhtanen, "Study of the elimination and distribution of radiolabel following oral administration of [¹⁴C]IKF-916 to Sprague-Dawley rats" (incl. Report Amendment No. 1), Ricerca, Inc., Painesville, OH, 9/21/99. Laboratory Study # 6920-96-0201-AM-001-001. Groups of 3 to 5 rats/sex were gavage-treated (in 0.75% methylcellulose suspension) with variations on dose level, label placement, and termination times, in the primary single-dose segment of the rat metabolism studies. Dose levels were low (0.5 mg/kg) or high (1000 mg/kg). The test article was either phenyl-labeled ([¹⁴C-Bz]IKF-916), or imidazole-labeled ([¹⁴C-Im]IKF-916). Rats were killed near to the time of peak blood levels (0.5 hr or 0.25 hr for low and high dose groups, respectively), at a variable intermediate time (5.5 hr or 10 hr for low and high dose groups, respectively); or at 24 hr and 168 hr, each for low and high dose regimens). Investigators measured urinary and fecal excretion and tissue distribution over time. A previous study had found only traces of labeled carbon in cage air samples, so air was not sampled here. Major metabolites in urine and feces were identified. There were no significant products of cleavage between phenyl and imidazole substituents. Most of administered label was absorbed following low dose treatment, although 16 to 20% of administered label was found as unchanged a.i. in feces. Males given low dose treatment excreted more label in urine than in feces (about 2:1), whereas in females the ratio was about 1:1. This difference probably reflects a greater excretion of absorbed material in females via the bile compared to males (see Record No. 220234). High dose level was very poorly absorbed, evidenced by 86-87% of administered label being found in feces as unchanged a.i. All identified metabolites displayed hydrolytic cleavage of the N,N-dimethylsulfonamide group away from the imidazole ring. Of remaining substituents, the methyl group on the phenyl ring was either oxidized to a carboxylic acid (the major urinary metabolite), or conjugated by GSH and further modified to form a series of metabolites. The major two of these metabolites were α -(methylsulfinyl)-*p*-tolyl and α -(methylsulfonyl)-*p*-tolyl derivatives, both of which were primarily limited to urine of low dose females. Label clearance from blood and other tissues was rapid. In low dose rats there were typically about 10-fold reductions from peak concentrations at 0.5 hr after dosing to the next sampling at 5.5 hr after dosing. Acceptable (as the core study of a series of related studies). Aldous, 7/18/06.

52995-0044 220235 Huhtanen, K. L. and M. C. Savides, "A pharmacokinetic study of radiolabel in blood following oral administration of [¹⁴C]IKF-916 to Sprague-Dawley rats" (incl. Report Amendment No. 1), Ricerca, Inc., Painesville, OH, 9/23/99. Rats were implanted with jugular cannulae 3 to 8 days prior to receiving single gavage treatment with either phenyl-labeled ([¹⁴C-Bz]IKF-916), or imidazole-labeled ([¹⁴C-Im]IKF-916) cyazofamid. Five rats/sex/dose were dosed with either 0.5 or 1000 mg/kg [¹⁴C]IKF-916, with each of the two label placements. Investigators sampled blood from rats at 0.25, 0.5, 1, 1.5, 3, 6, 8, 24, 48, and 72 hr after dosing. Study objective was strictly to study kinetics based on radiolabel in blood. Investigators found peak blood levels at 0.5 hr or 0.25 hr for low and high dose groups, respectively. Beginning at about 3 hr after dosing (considered to be the end of the distribution

phase), investigators determined the biological $t_{1/2}$ as affected by sex, dose, and radiolabel position. Sex and label position had no measurable effect on $t_{1/2}$, however high doses delayed clearance. Estimated $t_{1/2}$ values were about 5 hr or 10 hr for low and high dose groups, respectively. Information from this study was used to establish post-treatment termination times for the primary metabolism study (Record No. 220236, above). Aldous, 7/27/06.

52995-0046 220237 McFadden, J. J., and M. C. Savides, "Study of the elimination and distribution of radiolabel following multiple oral administrations of [$^{12}\text{C}/^{14}\text{C}$]IKF-916 to Sprague-Dawley rats" (incl. Report Amendment No. 1), Ricerca, Inc., Painesville, OH, 9/21/99. Laboratory Study #: 7526-98-0062-AM-001-001. The primary objective was to assess the effects of pre-treatment on metabolic fate of cyazofamid, compared with the primary single-dose study [Record No. 220236 (Laboratory Study # 6920-96-0201-AM-001-001)] as the reference study. Groups of 2 rats/sex/termination time were gavaged-treated (in 10 ml/kg 0.75% methylcellulose suspension) with non-radioactive cyazofamid (IKF-916, 0.5 mg/kg/day) for 14 consecutive days. On day 15, each rat received 0.5 mg/kg of phenyl-labeled cyazofamid ([^{14}C -Bz]IKF-916) orally. There were 3 termination times (0.5 hr, 24 hr, and 168 hr), thus this study utilized only a total of six rats/sex (hence offering minimal statistical power). Investigators measured urinary and fecal excretion and tissue distribution over time. Major metabolites in urine, feces, and plasma were evaluated strictly by radioactivity elution profile. There appeared to be slight increases in the percent administered dose being eliminated in urine, and the duration of urinary excretion appeared to be slightly longer after multiple dosing than after a single treatment (residual urinary excretion became trivial after 24 hr with single dosing, compared to after 48 hr with multiple dosing). In males there was a parallel longer retention of label in the g.i. tract in repeat-dose rats. In both sexes there appeared to be a slight increase (about 10%) in relative amount of label in urine and correspondingly lower amount in feces following multiple dosing. Residue levels in tissues in this study at 24 hr were marginally higher than reported for single low dose males and females, however this probably relates to slower passage through the alimentary tract. Residue levels at 168 hr in multiple dose rats were comparable to levels in single low dose males and females. Urinary metabolite profiles were comparable to the single dose study. Fecal metabolite profiles contained more notable extractable peaks than were obtained in the single dose study, nevertheless the predominant peak with or without pre-treatment was the a.i., and major resolvable peaks had retention times consistent with recognized peaks observed in urine in the single dose study. In summary, the small observed apparent differences between metabolic activity associated with pre-treatment for 2 weeks with low doses of cyazofamid (and its vehicle) do not represent novel metabolic patterns, and some of the apparent differences may be a function of small sample size. Useful supplementary data. Aldous, 7/18/06.

52995-0043 220234 Huhtanen, K. L. and M. C. Savides, "Study of the biliary elimination of radiolabel following oral administration of [^{14}C]IKF-916 to Sprague-Dawley rats" (as amended), Ricerca, Inc., Painesville, OH, 9/23/99. Laboratory Study #: 6823-96-0087-AM-001-001. This study is supplementary to the major single-dose metabolism study (DPR Document No. 52995-0045, Record No. 220236, Laboratory Study # 6920-96-0201-AM-001-001.) Generally 3 biliary-cannulated rats/sex/group were dosed once by gavage with either low or high dose level of cyazofamid (0.5 or 1000 mg/kg): either phenyl-labeled ([^{14}C -Bz]IKF-916), or imidazole-labeled ([^{14}C -Im]IKF-916). The only strong peak found in urine (50% of administered dose in M and 38% in F) was CCBA. Virtually all label in the feces of low-dose cannulated rats was the parent cyazofamid. Bile accounted for 12-22% of administered dose (M) or 29-39% of administered dose (F). Bile HPLC profiles were rather complex, displaying mostly rather polar components. Investigators justifiably concluded in the footnotes to these pages that structures were "predominantly catabolic products of the glutathione conjugate of CCIM" (CCIM is 4-chloro-5-p-tolylimidazole-2-carbonitrile). The benzoic acid metabolite, CCBA, was also a significant component of bile (about 4% of administered dose in M and F). Complex HPLC profiles of bile

extracts were progressively simplified to a few major peaks upon treatment with glucuronidase and acidification. A low-dose male bile profile after such treatment yielded 19% of label in bile as CHCN, and a slight increase over the pre-processing levels of CCBA. CHCN is CCIM with the methyl group on the phenyl ring oxidized to a hydroxymethyl. Together CHCN and CCIM comprised 46% of the bile extracts thus treated, and nearly all of the balance of radiolabel was found in two peaks of relatively polar material (indicated by quite low R_f values). Thus it appears that conjugation of bile products to glucuronides is a quantitatively important process, in addition to conjugation by glutathione. It was noted in the core metabolism study (Record No. 220236) that $\text{CH}_3\text{SO}_2\text{-CCIM}$ and $\text{CH}_3\text{SO-CCIM}$ (two products of glutathione addition and subsequent modification) were abundant in urine of non-cannulated females; however cannulated rats of either gender in this study yielded no common urinary metabolites other than CCBA. This suggests that these two metabolites were biliary glutathione derivatives. This is an acceptable supplementary study, however this review noted that investigators chose not to attempt to quantify the minor polar metabolites. Aldous, 7/18/06.

52995-0042 220233 Huhtanen, K. L. and M. C. Savides, "Pilot study of the routes of elimination of radiolabel following oral administration of [^{14}C]IKF-916 to Sprague-Dawley rats," Ricerca, Inc., Painesville, OH, 4/21/98. Laboratory Study #: 6617-95-0274. This was the pilot for Record No. 220236. Aside from reporting very low levels of $^{14}\text{CO}_2$ in the carbon trap collections of expired air (less than 0.01% to about 0.03% of administered dose), there is no unique information in this report, and no DPR worksheet is needed. Aldous, 7/18/06.

52995-0047 220238 Sakai, A., Y. Tada, and T. Kanza, "*In vitro* metabolism of IKF-916 and CCIM in blood and stomach contents," Ishihara Sangyo Kaisha, Ltd., Kusatsu, Shiga, Japan, 11/11/99. Investigators tested metabolism of [^{14}C]IKF-916 (i.e. cyazofamid; 0.5 mg/kg, phenyl label) or an equimolar amount of phenyl labeled [^{14}C]CCIM, each in rats' blood or rat stomach contents. Blood was sampled at times 0, 15, 30, and 60 min. Stomach contents were sampled at 0 and 60 min. About 33% of cyazofamid was degraded in blood in one hour, nearly entirely to CCIM. CCIM in blood was unchanged in these conditions. Stomach contents had no apparent effect on either cyazofamid nor on CCIM. Aldous, useful data: no DPR worksheet. 7/20/06.

52995-0048 220239 Kanza, T., "*In vitro* metabolism of CCIM in S9 fraction," Ishihara Sangyo Kaisha, Ltd., Kusatsu, Shiga, Japan, 11/13/99. Either low or high levels of CCIM (0.27 $\mu\text{g/ml}$ and 2.7 $\mu\text{g/ml}$, respectively) were incubated with an S-9 mix (including cofactors) for up to 5 minutes, with aliquots taken at 1-min intervals. In the low-dose mixture, only 6% of the original CCIM concentration remained after 1 minute, and non-detectable levels were observed thereafter. CHCN was the major metabolite after 1 minute (89% of administered label). Over time, the percent of CHCN went down as the percent of CCBA rose, eventually to 13% of administered dose after 5 min. Several unidentified metabolites were noted, which consisted of 10% of administered dose by 5 min. The higher level of CCIM eventually yielded 94% of label as CHCN, however it took 5 minutes to reduce CCIM to non-detectable levels. Aldous, useful data: no DPR worksheet, 7/20/06.

52995-0049 220249 Kishimoto, D., A. Sakai, Y. Tada, and T. Kanza, "*In vitro* study to identify metabolites absorbed through the intestinal mucosa after incubation with IKF-916 and CCIM," Ishihara Sangyo Kaisha, Ltd., Kusatsu, Shiga, Japan, 11/15/99. Investigators prepared emptied rat intestine as 8-cm sections originating distal to the common bile duct. These gut sacs were instilled with 500 μl of methylcellulose suspension containing 0.05 mg/ml of cyazofamid or a molar equivalent of CCIM. Sacs were ligated on both ends. Sacs were maintained in Krebs-Henseleit buffer bubbled with 95% O_2 /5% CO_2 at 37 $^\circ\text{C}$ for 1 hr. Investigators then evaluated label concentration and metabolite distribution on the "serosal" side of the preparation (the buffer around the sac, and the water/acetonitrile rinse of the external surface of the sac), as well

as the “mucosal” side (sac luminal contents, plus acetonitrile rinse of the interior of the sac). Apparent absorbed label (serosal buffer plus rinse) was 4-fold lower after treatment with cyazofamid than after CCIM treatment (2.6 % of administered dose vs. 10.4% of administered dose, respectively). On the mucosal side, 92% of administered dose was found in the luminal content following cyazofamid dosing, compared with 77.8% of CCIM administered. Also, less label was recovered upon rinsing the mucosal surface after cyazofamid treatment (5.3% of administered dose) than after CCIM treatment (11.2% of administered dose). Most of mucosal content and rinse label following cyazofamid treatment was parent cyazofamid. Also, much of the label content on the serosal side of the sac following cyazofamid treatment was also cyazofamid (50% of buffer label, and 84% of rinse label). The most common recovered labeled material other than cyazofamid after cyazofamid treatment was CCIM. CCIM was by far the major recovered labeled material in all mucosal and serosal samplings after CCIM treatment. Small amounts of CCBA plus various unidentified metabolites were also seen after treatment with either material. Aldous, useful data: no DPR worksheet, 7/20/06.

52995-0050 220250 Murray, M. D. and M. C. Savides, “Comparative metabolism of [¹⁴C]-IKF-916 and [¹⁴C]-CCIM in rats,” Ricerca, Inc., Painesville, OH, 12/13/99. Ricerca Document No. 11334-1. Five male Sprague-Dawley rats/group were dosed with either [¹⁴C]-IKF-916 (i.e. cyazofamid; 0.5 mg/kg, phenyl label) or an equimolar amount of phenyl labeled [¹⁴C]-CCIM. Rats were killed after 30 min, at which time blood, liver, and stomach (with contents) were collected and assayed for test articles and major metabolites. Stomach contents constituted 20% or 24% of administered dose following cyazofamid or CCIM dosing. Virtually all stomach contents following cyazofamid administration was parent IKF-916: the only other identified labeled material in stomach constituted less than 1% administration dose, namely CCIM (see report Table 4). Stomach contents of CCIM-treated rats contained CCIM as the only notable labeled constituent. Cyazofamid administration led to only about 0.01% of dose being found as parent compound in the liver, and no parent material was found in plasma. In contrast, following CCIM treatment, there were substantial levels of CCIM in plasma (6.5% of administered dose) and liver (2.0% of administered dose). CCIM-treated rats also had measurable amounts of metabolic products of oxidation of the benzyl carbon in plasma and liver, particularly CCBA and lesser amounts of CHCN. These concentrations were commonly about 20-fold higher than those following equimolar amounts of cyazofamid. Results suggest that metabolism of cyazofamid began with degradation to CCIM, and that CCIM was much more readily absorbed than cyazofamid under these test conditions. Aldous, useful data: no DPR worksheet. 7/20/06.

Note: Rat pharmacokinetic and metabolism study data are sufficient to fulfill data requirements.

SUBCHRONIC STUDIES

Rat 4-Week Dietary Toxicity Study

0021, 220211; “IKF: 4-Week Dose Range Finding Study in Rats” (Nakashima, N., The Institute of Environmental Study, Tokyo, Japan, Laboratory Project ID IET 95-0077, 03/23/99).

IKF-916 (Lot no. 9506, purity = 97.3%) was admixed to the basal diet and fed to 6 Fischer (F344/DuCrj) rats per sex per dose at dose levels of 0, 50, 500, 5000, or 20000 ppm (0, 3.772, 38.47, 370.4, and 1488 mg/kg/day, respectively, for males and 0, 3.644, 37.12, 389.3, and 1535 mg/kg/day, respectively, for females) for 4 weeks. No mortalities occurred. No clinical signs were observed. No effect on body weight was observed. Treatment-related increases in serum mean alkaline phosphatase, protein, globulin, albumin, and glucose levels were observed in males at 5000 and 20000 ppm and a treatment-related decrease in the triglyceride level was observed in males at 20000 ppm. Treatment-related increases in mean relative kidney weight in males at 20000 ppm and in mean relative liver weight in males at 5000 and 20000 ppm were observed. Microscopic examination revealed a treatment-related increase in the number of basophilic tubules in the kidneys in males at 5000 and 20000 ppm. **No adverse effects.** NOEL

(M) = 38.47 mg/kg/day (500 ppm) based on an increase in the number of basophilic tubules in the kidneys, NOEL (F) = 1535 mg/kg/day (20000 ppm) based on no effects at the highest dose tested. **Supplemental study** (only 6 animals per dose level were used, the test animals were treated for only 4 weeks, and no ophthalmological examinations were conducted). (Corlett, 02/09/06)

Rat 13-Week Dietary Toxicity Study

0022, 220212; "IKF-916: 13-Week Oral Subchronic Toxicity Study in Rats" (Nakashima, N., The Institute of Environmental Study, Tokyo, Japan, Laboratory Project ID IET 95-0078, 03/23/99). 821. IKF-916 (Lot no. 9506, purity = 95.5%) was admixed to the basal diet and fed to 12 Fischer (F344/DuCrj) rats per sex per dose at dose levels of 0, 10 (males only), 50, 500, 5000, or 20000 (females only) ppm (0, 0.597, 2.906, 29.51, and 294.5 mg/kg/day, respectively, for males and 0, 3.303, 33.32, 337.6, and 1359 mg/kg/day, respectively, for females) for 13 weeks. No mortalities occurred. No treatment-related clinical signs were observed. No biologically-significant effects on body weight were observed. A treatment-related increase in serum mean chloride level and treatment-related decreases in total cholesterol and triglyceride levels were observed in males at 5000 ppm. Urinalysis revealed a treatment-related increase in mean urine volume and a treatment-related increase in protein in males at 5000 ppm. Treatment-related increases in mean relative kidney weight in females at 5000 and 20000 ppm and in mean relative liver weight in females at 20000 ppm were observed. Microscopic examination revealed a treatment-related increase in the number of basophilic tubules in the kidneys in males at 5000 ppm. **No adverse effects.** NOEL (M) = 29.51 mg/kg/day (500 ppm) based on an increase in the number of basophilic tubules in the kidneys, NOEL (F) = 33.32 mg/kg/day (500 ppm) based on an increase in mean relative kidney weight. **Acceptable.** (Corlett, 02/16/06)

Dog 13-Week Oral Toxicity Study

0023, 220213; "A 13-Week Oral Toxicity Study in Dogs with IKF-916 (Includes Report Amendment No. 1)" (Savides, M.C. et al., Department of Toxicology, Ricerca, Inc., LLC, Painesville, OH, Document Numbers 6898-96-0141-TX-002 and 6898-96-0141-TX-002-001, 03/21/01). 821. Technical grade IKF-916 (Lot no. 9506, purity = 96.2%) was administered orally by capsule(s) daily to 4 beagle dogs per sex per dose at dose levels of 0 (empty capsules), 40, 200, or 1000 mg/kg/day for 13 consecutive weeks. No mortalities occurred. No treatment-related clinical signs were observed. No effects on body weight were observed. Hematology and clinical chemistry investigations revealed no biologically significant effects. Urinalysis revealed no treatment-related effects. Organ weight data revealed no biologically significant effects. Necropsy revealed no treatment-related abnormalities. Microscopic examination revealed no histopathological findings. **No adverse effects.** NOEL (M/F) = 1000 mg/kg/day based on no effects at the highest dose tested. **Acceptable.** (Corlett, 03/22/06)

Rat 28-Day Repeated Dosing Dermal Toxicity Study

0024; 220214; "A 28-Day Repeated Dose Dermal Toxicity Study in Rats with Technical IKF-916" (O'Meara, H.M. and Watson, M., Bayer AG, Department of Toxicology, Ricerca, Inc., LLC, Painesville, OH, ISK Document Number 6717-96-0023-TX-002, 12/05/97). 822. Technical IKF-916 (Lot Number 9506, purity = 95.5%) was moistened with deionized water and applied to the clipped dorsal skin of 5 Sprague Dawley [CrI:CD®(SD)BR] rats per sex per dose at dose levels of 0 (deionized water), 250, 500, or 1000 mg/kg/day for 6 hours per day for 28 consecutive days. No mortalities occurred. No treatment-related clinical signs and no skin effects at the test site were observed. No effects on body weight were observed. Hematological and clinical chemistry investigations revealed no treatment-related effects. No treatment-related changes in organ weights were observed. Necropsies and microscopic examinations revealed no treatment-related effects. **No adverse effects.** NOEL (M/F, systemic and skin) = 1000 mg/kg/day (based on no effects at the highest dose tested). **Acceptable.** (Corlett, 03/14/06)